Al-Salihi, S. A. A., Scott, T. A., Bailey, A. M., & Foster, G. D. (2017). Improved vectors for Agrobacterium mediated genetic manipulation of Hypholoma spp. and other homobasidiomycetes. *Journal of Microbiological Methods*, *142*, 4-9. https://doi.org/10.1016/j.mimet.2017.08.014

Supplementary data

Figure S1: Construction diagram for plasmids used for GFP expression in both *Hypholoma spp.*

Figure S2: The *gpd* gene sequences of *H. sublateritium* (JGI protein ID = 45836).

Table S1: List of primers used in yeast homologous recombination, plasmids verification and *Hypholoma spp*. transformants.

Figure S3: *H. fasciculare* colony diameter on media supplemented with different concentrations of hygromycin.

Figure S4: *H. sublateritium* sensitivity to different concentrations of hygromycin.

Figure S5: Average number of transformants obtained in the presence of various concentrations of acetosyringone for both *H. fasciculare* (Hfas) and *H. sublateritium* (Hsub).

Figure S6: Average number of transformants obtained using different optical density (OD_{600}) of *Agrobacterium* preculture for both *H. fasciculare* (Hfas) and *H. sublateritium* (Hsub).

Figure S7: PCR amplicons of 10 randomly selected transformants of *H. sublateritium.*

Figure S8: PCR amplicons of 10 randomly selected transformants of *H. fasciculare*

Figure S9: Confirmation of correct construction of plasmids pCAM-*hph*-Hs*gpd*GFP and pCAM-*hph*-Hs*gpd*iGFP

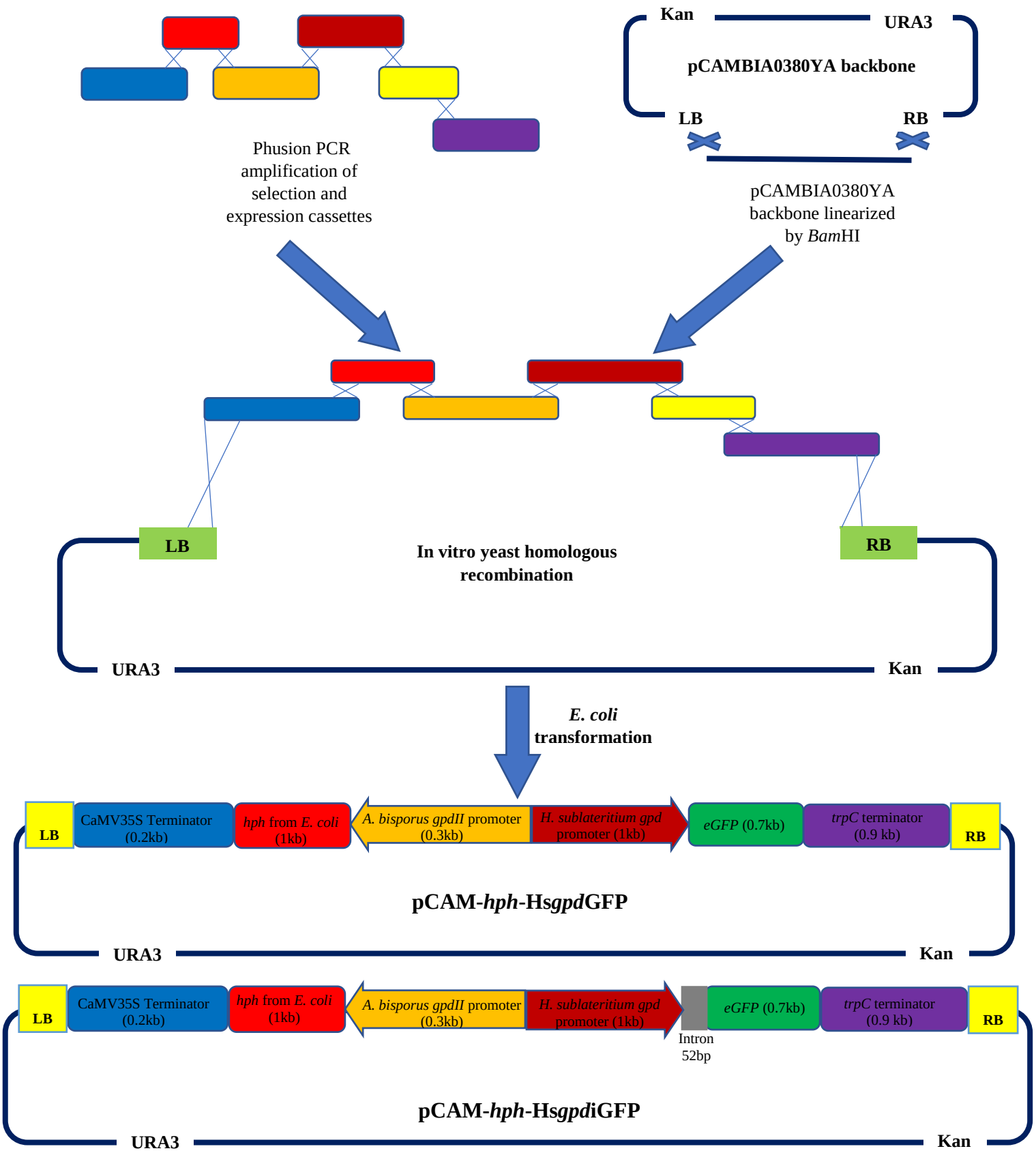

Figure S1: construction diagram for plasmids used for GFP expression in both *Hypholoma spp.*
A- plasmid pCAM-*hph*-Hs*gpd*GFP (using pCAMBIA0308YA backbone) carrying hygromycin under *A. bisporus gpdII* promoter, and CaMVT terminator, while GFP derived under *H. sublateritium gpd* promoter, and *A. nidulans trpC* terminator.
B- plasmid pCAM-*hph*-Hs*gpd*iGFP (using pCAMBIA 0308YA backbone) carrying hygromycin under *A. bisporus gpdII* promoter, and CaMVT terminator, while GFP derived under *H. sublateritium gpd* promoter+ 1st intron, and *A. nidulans trpC* terminator.

GTACTTACTAGCCCATCTTTGGCTCTATCGTTCGGCTCGGCGACCATTTTCCCAAAATATTTCCTGGTA
CGACAACGACTGGGGATGCCTTAATAGTGGTTACGGCCATACCTGACACCAGACGCCAAAGCAAGAGTC
AGATAAATATAATCAATTTCTCGTCGACTTGCTCTGGTCATCTTTTACATCCTAGTACGGTGGCAGGGC
ATACGTACTATGGTTGAGGGTAAATCATGCCGGTAGAGTATCACACGGAAAGTGCCAGTACTACCTAGT
ACAAACCTAGTACGTGATTCTGTGAAATGACCAATACAATTGGATGCCGCGATTGTACTGCGATAACAA
TGCAGTTACCGAATATACTCGAGGCGCAAGGCCAGAAGCCATCTTTCTCTTCATGATCGATGTAATATT
TCACTATTCGACCGCTACGGTACACTTTGGCGAGAAGTGTCTAGAACAAGGGTTGCAATTGCAGCGACC
TAAAACTGTGCAAACATCGCGTAGATTCGACGCGAATGTACAGCCCGGACTGTACGATCTATTTTTGGA
GCTGCTCAGGCCTGCTCCTTCGGAGTCCTTGGAAGCGCCTGCCAAATGTTCTAGGCCCATTCCGCGTCC
CTGGCGCCGCACGTTGGAGGTTGTTGGAGGGCG**CAAT**CACTGATGTCGTCATGGATTCTTACATTGGAT
ATTGGGGGGAAAGTGCGCCCCAAACTTTCCCCCTAGCTTACG**CCTTTCCTTC**ATTACAGGATGTTAGAG
ACTAGCCAACGTTCCACTCCTTACCAGTACCGATTATAAACAGCCTTCTAACTGCTCACAAAGCTGGGGG
GATATGACTCAAGATTACAGAGGCCTGTATTTTTGTCAGCGATGGATTTG**TATA**CGCATCTCGAATGCA
ACGAGATGCAGCGCGCCTGGGCCCAGAGCGAGCGGCCGAGTGAAATGTGTTTCCTTTTGCGGCCGGTGA
GCCGCATTTATCCACCAAACCAGTTCGATGCTGTCATTTGTCAGGCAATATGCAGCGCGACCCAACTCG
GCGCCGCGCGCTGTGATTGGCCAACAGCGCCTCTCCATCGATAGATAATCGTTCCGGCGGATCTATATA
AGTGGGCCGCATCGGACTTTCGCTCTTCACGCATTTTTTGCATCCTCCCCAACCACCACATCCCCAACA
CACTTTCAGA**ATG**gtgagtcgcctcgccctgtcttagttaccattgtgctcatcgccgccgcc
agGTCAACGTCGGTATCAACGGgtaagtactgtcgaacaactgTTTgacaatatgactaagttac
gttcaaaatttcagtttcggtgcgtattcatgcagatattgtcgcgctgaaactcacgatgag
cAGGTCGTATCGGCCGTATCGTCTTCCGTAACGCTCTTGAGGTCCAGGGTCTCGATGTCGTCGCCATCA
ACGAgtgcgtcgtttttcgttgctggttttcatttttaatgtgcctgcagCCCCTTCATTGA
CCTTGACTACATGGTCTACATGTTCAAGTACGATTCCGTTCACGGACGCTTCAAGGGAACCGTCGAGGC
CAAGGACGGAAAGCTCGTCATCAACGGAAAGCCCGTCTCTGTCTTCGCTGAGCGCGACCCCAGTGCCAT
CCCTTGGGGATCGGCCGGCGCCGACTACGTCGTTGAGGCCACTgtaagttatttttgacgcgtatta
tttccatggactctcatcgctaatgtcggactttagGGTGTCTTCACAACCACCGAGAAgtaggt
cttctcttgattgcattccgcatgtgctaacatccatacccagGGCCTCCGCCCACTTGAAGGGT
GGTGCCAAGAAGGTCGTTATCTCCGCCCCCTCCGCTGATGCACCCATGTTCGTATGCGGTGTCAACCTG
GAGGCTTACGATCCCAAGTACAAAGTTgtgagtagcgattcaacccgcgtcgttatctatgacga
catctgatcttatctctgccgccaatgcgattagATCTCCAACGCCTCCTGCACCACCAACTGCCT
CGCCCCCCTCGCTAAGATCATCAACGACAACTTCGGAATTGTCGAGGGCTTGATGACCACCGTCCATGC
CACCACTGCCACCCAAAAGACCGTCGATGGTCCCTCCCACAAGGACTGGCGTGGAGGACGTTCCGTCAA
CAACAACATCATCCCCTCCTCCACTGGTGCTGCCAAGGCCGTCGGAAAGGTCATCCCCGCCCTCAATGG
AAAGCTCACgtacgtgatcttctaaaaaactgaattatccattttgtgctaatgcgcctccaa
cagGGGCCTTGCATTCCGTGTCCCCACTCTCGATGTCTCCGTCGTCGATCTCGTTGTCCGCCTTGAAAA
GGGTGCCTCCTACGAGGAGATCAAGGCCGTCGTCAAGGCCGCCTCTGAGGGCGAGTACAAGGGTATCGT
CGACTACACCGATGAGCTCGTCGTCTCCACCGATTTCATCGGCAGCCACGCATCCTCGATCTTCGACGC
CAACGCCGGAATCCAGCTGAGCCCTAACTTCGTCAAGCTCATCTCCTGGTACGACAACGAGTGGGGTTA
CTCCCGCCGCGTGTGCGACCTCATCAACTACGTCGCTGCCAAGGACGCCGCCGCCGGCCTC**TAA**

Figure S2: The *gpd* gene sequences of *H. sublateritium* (JGI protein ID = 45836).
Grey sequences = 1Kb upstream of *gpd*.
Sequences in boxes = promoter elements (TATA box, CT stretch and CAAT).
Underlined sequences = coding region.
Lower case sequences = non-coding regions (predicted introns).

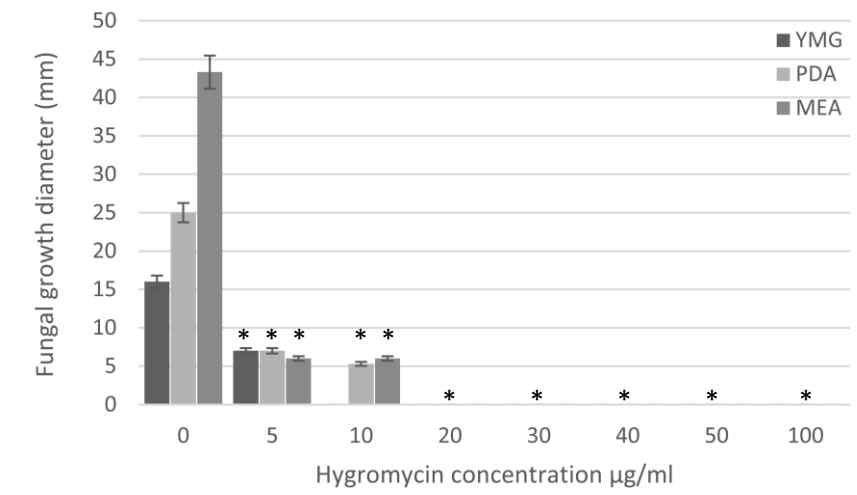

Figure S3: *H. fasciculare* colony diameter on media supplemented with different concentrations of hygromycin (µg/ml) on YMG, PDA and MEA media after 2 week of incubation. YMG = yeast malt glucose agar, PDA = potato dextrose agar and MEA = malt extract agar. * indicates significant difference compared to non-supplemented media determined by factorial ANOVA and confirmed by Dunnett's post-hoc test where $P<0.05$.

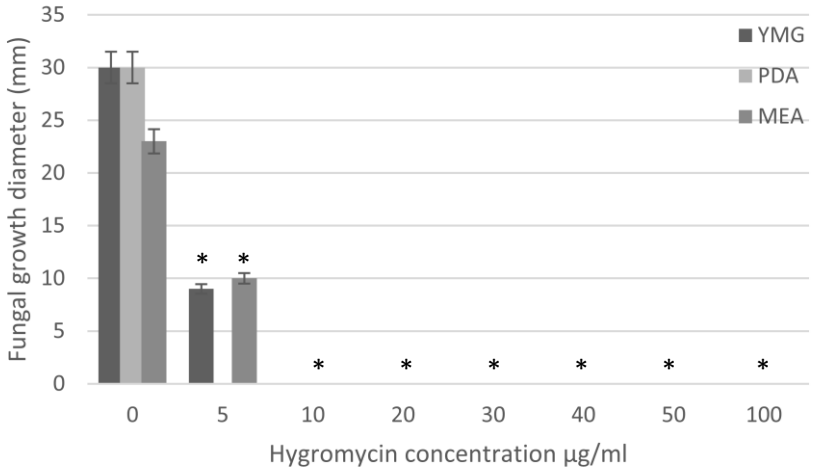

Figure S4: *H. sublateritium* sensitivity to different concentrations of hygromycin (µg/ml) on YMG, PDA and MEA media after 2 week of incubation. YMG = yeast malt glucose agar, PDA = potato dextrose agar and MEA = malt extract agar. * indicates significant difference compared to non-supplemented media determined by factorial ANOVA and confirmed by Dunnett's post-hoc test where $P<0.05$.

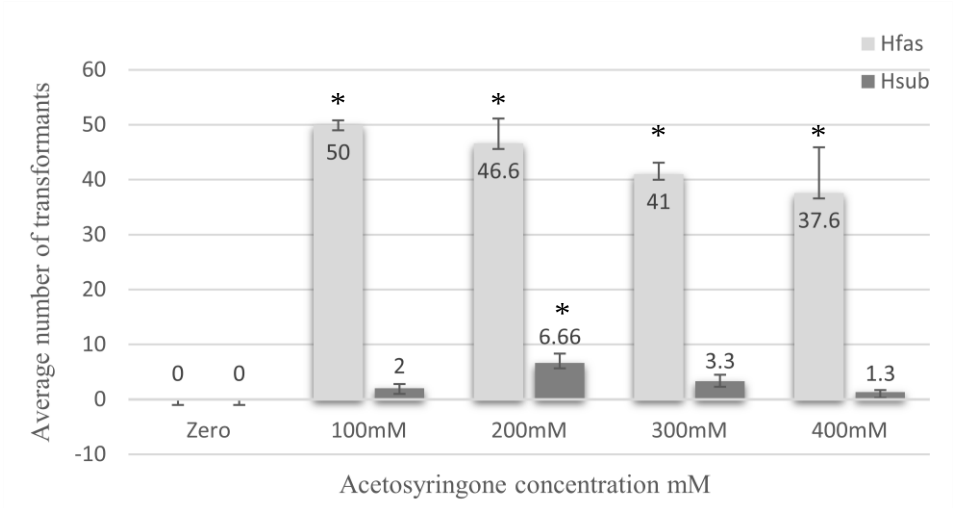

Figure S5: Average number of transformants obtained in the presence of various concentrations of acetosyringone for both *H. fasciculare* (Hfas) and *H. sublateritium* (Hsub). * indicates significant difference compared to non-supplemented media determined by factorial ANOVA and confirmed by Dunnett's post-hoc test where $P<0.05$.

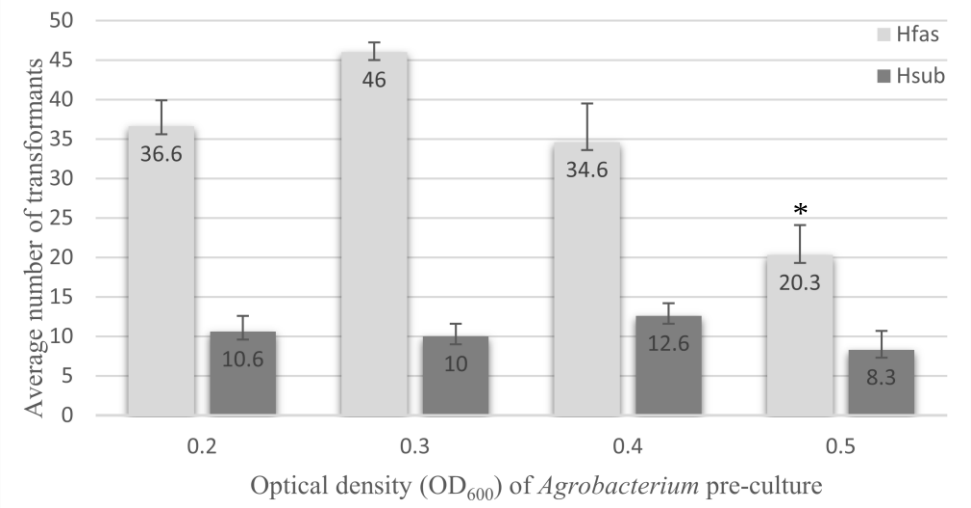

Figure S6: Average number of transformants obtained using different optical density (OD_{600}) of *Agrobacterium* preculture for both *H. fasciculare* (Hfas) and *H. sublateritium* (Hsub). * indicates significant difference compared to other OD_{600} determined by factorial ANOVA and confirmed by Tukey honest significant difference (HSD) post-hoc test where $P<0.05$.

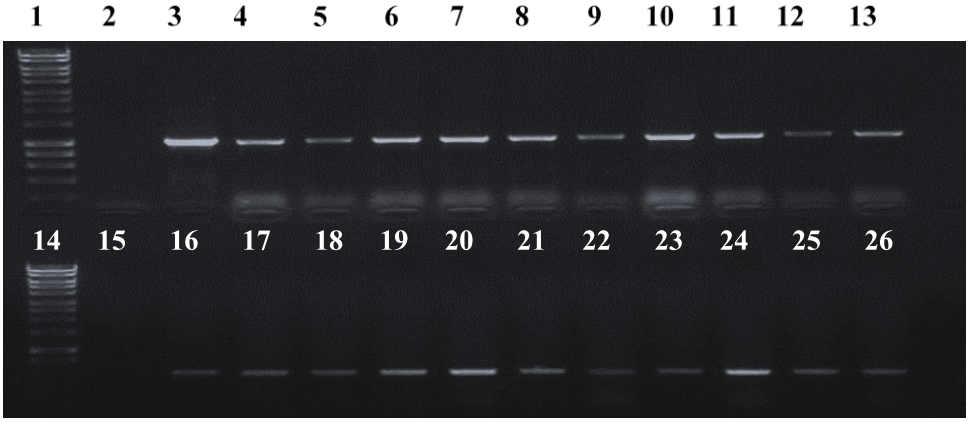

Figure S7: PCR amplicons of 10 randomly selected transformants of *H. sublateritium.* Lane 1 and 14 are Hyperladder I, Lane 2 -ve control (SDW), Lane 3 pBGgHg plasmid as PCR template (+ve control) showing *hph* amplicon of 1kb, Lanes 4-13, 10 randomly selected transformants of *H. sublateritium* showing the 1kb *hph* amplicon, Lane 15 *H. sublateritium* wild type with *hph* primers giving no detectable amplicon.. Lanes 16-26 showing ITS amplification, Lane 16 shows the wild-type whilst lanes 17-26 are of the same ten selected transformants, all showing the expected ITS amplicon.

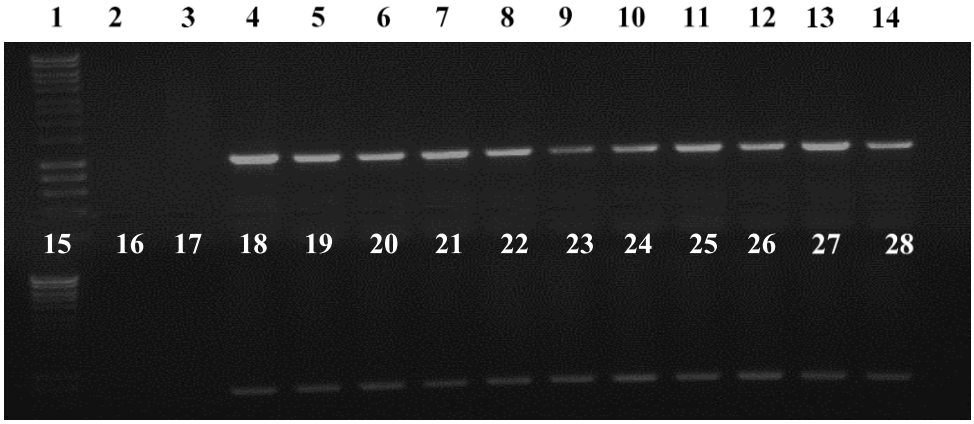

Figure S8: PCR amplicons of 10 randomly selected transformants of *H. fasciculare.* Lane 1 and 15 are Hyperladder, Lane 2 and 16 are -ve control (SDW), Lane 3 *H. fasciculare* wild type showing no amplicon for *hph* gene, Lane 4 PCR from pBGgHg plasmid (+ve control) showing 1kb *hph* amplicon, Lane 5-14, transformants showing the *hph* amplicon. Lane 17 pBGgHg plasmid (-ve control) showing no amplicon for ITS, Lane 18 *H. fasciculare* wild type (+ve control) showing ITS amplification, Lanes 19-28 same selected transformants showing the expected ITS amplicon.

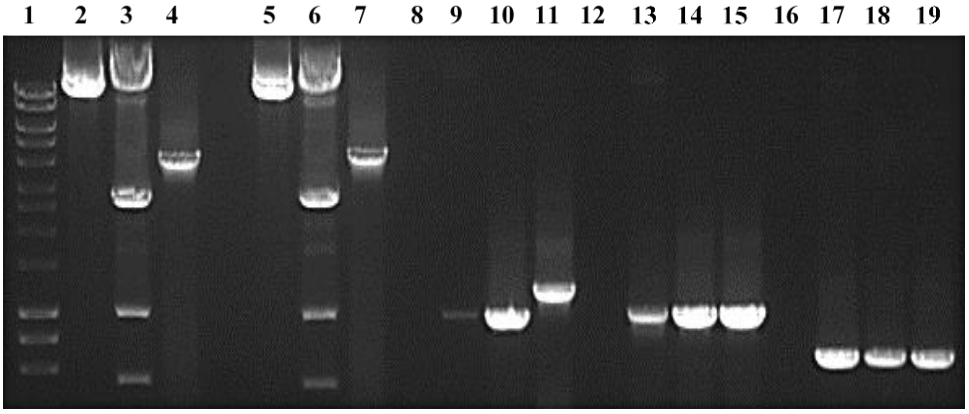

Figure S9: Confirmation of correct construction of plasmids pCAM-*hph*-Hs*gpd*GFP and pCAM-*hph*-Hs*gpd*iGFP.

Lane 1 hypper ladder.
Lane 2 pCAM-hph-Hs*gpd*GFP miniprep.
Lane 3 pCAM-hph-Hs*gpd*GFP miniprep digested with *Eco*RI enzyme showing expected fragments size (1006, 508, 2561 and 9639bp).
Lane 4 whole insertion (hygromycin, Hs*gpd* promoter and GFP) using pCAM-*hph*-Hs*gpd*GFP as DNA template, showing the expected size (±4090bp).
Lane 5 pCAM-*hph*-Hs*gpd*iGFP miniprep .
Lane 6 pCAM-*hph*-Hs*gpd*iGFP miniprep digested with *Eco*RI enzyme showing expected fragments (1006, 508, 2799 and 9639bp).
Lane 7 whole insertion (hygromycin, Hs*gpd* promoter +1st intron and GFP) using pCAM-*hph*-Hs*gpd*iGFP as DNA template, showing the expected size (±4327bp).
Lane 8, 12 and 16 negative controls (deionized water), showing no amplification
Lane 9 *H. sublateritium gpd* promoter gene using *H. sublateritium* gDNA as DNA template (+ve control).
Lane 10 *H. sublateritium gpd* promoter using pCAM-*hph*-Hs*gpd*GFP as DNA template showing the expected size.
Lane 11 *H. sublateritium gpd* promoter +1st intron using pCAM-hph-Hs*gpd*iGFP as DNA template showing the expected size.
Lane 13 hygromycin resistance gene using pBGgHg as DNA template (+ve control) showing the size of *hph* gene.
Lane 14 hygromycin resistance gene using pCAM-*hph*-Hs*gpd*GFP as DNA template showing the expected size.
Lane 15 hygromycin resistance gene using pCAM-*hph*-Hs*gpd*iGFP as DNA template showing the expected size.
Lane 17 GFP gene using pBGgHg construct as DNA template showing the size of GFP.
Lane 18 GFP gene using pCAM-hph-Hs*gpd*GFP as DNA template showing the expected size.
Lane 19 GFP gene using pCAM-hph-Hs*gpd*iGFP as DNA template showing the expected size.